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# Viral Influence on Aquatic Bacterial Communities

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*Bacterial viruses, or bacteriophages, have numerous roles in marine systems. Although they are now considered important agents of mortality of bacteria, a second possible role of regulating bacterial community composition is less well known. The effect on community composition derives from the presumed species-specificity and density-dependence of infection. Although models have described the "kill the winner" hypothesis of such control, there are few observational or experimental demonstrations of this effect in complex natural communities. We report here on some experiments that demonstrate that viruses can influence community composition in natural marine communities. Although the effect is subtle over the time frame suitable for field experiments (days), the cumulative effect over months or years would be substantial. Other virus roles, such as in genetic exchange or microbial evolution, have the potential to be extremely important, but we know very little about them.*

For many years, viruses were not included in our conceptual models of how natural aquatic ecosystems function. This was primarily because they simply were not observed. However, it was learned about 10 years ago that viruses are highly abundant in such systems, roughly $10^{10}$ per liter, or about 10–20 times the bacterial abundance (Bergh *et al.*, 1989; Proctor and Fuhrman, 1990). Because viruses require a host to reproduce, this high abundance raised the obvious question of what organisms these viruses were infecting. Field studies showed that viral counts are much better correlated to bacterial counts than to chlorophyll. Therefore, although viruses infecting eukaryotes certainly occur and can be important components of food webs, the majority of native marine viruses are thought to infect bacteria or archaea (Fuhrman, 1999; see also Wilhelm and Suttle, 1999; Wommack and Colwell, 2000).

Abundance alone does not tell how active the viruses may be in infecting hosts. Viral activities in natural communities have been estimated by a few approaches, all yielding the conclusion that viral infection is an important component of bacterial mortality. The first approach is to observe and count infected host cells by transmission electron microscopy (Proctor and Fuhrman, 1990). Although only a small percentage of bacteria are visibly infected, that translates into 10% to 50% of the mortality (less for cyanobacteria). Viral activity has also been estimated *via* virus decay rates. Viral decay is caused by sunlight, enzymes, and other factors. These rates are typically a few percent per hour, and when one considers that the viruses are replaced by replication in infected host cells, these rates also translate into 10% to 50% of total bacterial mortality (reviewed by Fuhrman, 1999). Virus production rates have also been estimated directly, by three independent methods: (a) tritiated thymidine incorporation into viral DNA (Steward *et al.*, 1992), (b) dilution of fluorescently labeled viruses added to trace production and loss (Noble and Fuhrman, 2000), and (c) virus increase in samples filtered to reduce viral abundance (Wilhelm *et al.*, 2002). The results of such studies show that virus production is typically a few percent per hour, matching decay. This again translates into between 10% and 50% of bacterial mortality. (Note that the mortality estimates are not additive, but rather they represent estimates of the same process determined by a variety of approaches.) Mortality from viruses has been compared directly to that from protists, as estimated by loss of tritiated DNA over several generations, and the results are the same (Fuhrman and Noble, 1995). Overall, the evidence is strong that viruses can have a significant impact on mortality of bacteria and phytoplankton.

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Viruses impact nutrient cycling because viral activity leads to fragmentation of host organisms. Lysis products are dissolved substances and small particles that can be assimilated by heterotrophic bacteria. The result is a loop in the food web that has the net effect of oxidizing organic matter and regenerating inorganic nutrients. It also helps keep nutrients in the ocean top layer, where they can fuel production, instead of sinking out and being effectively lost from the euphotic zone (reviewed by Fuhrman, 1999).

One of the most interesting aspects of viruses in marine systems is their potential effect on community composition. This is expected because (1) viruses are generally specific to certain hosts, thought to be species or genus in most cases (Ackermann and DuBow, 1987), although some viruses have a broad range of hosts (Riemann and Middelboe, 2002); and (2) infection is density-dependent—that is, infection is more likely at high host densities due to increased contact rates. The presumed narrow host range and density dependence together suggest that viruses should preferentially infect the most common hosts: high abundance makes one more susceptible; rarity makes one less susceptible. This has the opposite effect compared to competitive dominance, and is called the "kill the winner" hypothesis (Thingstad and Lignell, 1997).

Thus, viruses might help to control monospecific blooms and increase diversity of host organisms in general. An important question is whether the effect is only replacement of sensitive strains with closely related virus-resistant strains, or whether completely different species can replace the infected ones. Probably both occur, but this process is not well studied. Although laboratory experiments suggest that development of resistance can help avoid infection, it is not so simple in natural systems where there are many species and complex interactions. Resistance is thought to have a cost in most instances and may allow other species to compete, as discussed in Fuhrman (1999) and Riemann and Middelboe (2002).

There have been some recent theoretical analyses of the interactions between virus infection and community composition. One set of models (Thingstad and Lignell, 1997) indicates that viruses can control host species composition even when they are responsible for a very small portion of the mortality. This is because, over several generations, even a small selective effect can have a major long-term influence. Even prior to the development of this theory, there were some relevant field studies. Waterbury and Valois (1993) examined cultivable marine *Synechococcus* and their viruses from near Woods Hole, Massachusetts. Despite a low reported viral impact on mortality, the *Synechococcus* strains dominant at any given time tended to be resistant to co-occurring viruses. Thus, viruses seemed to influence the strain composition, but the same genus persisted throughout the study period.

We have asked if the Waterbury and Valois (1993) result from cultivable *Synechococcus* can be generalized to other bacteria—that is, will variety of closely related strains persist over long periods? Perhaps the *Synechococcus* story is not applicable to other groups, because this genus may occupy a unique photosynthetic niche that another marine genus cannot fill in that environment.

Our own experiments on viral effects have included a few kinds of experiments. One type is growth of mixed bacterial communities in the presence and absence of viruses. The protocol for growing such communities was developed several years ago (Wilcox and Fuhrman, 1994). The inoculum we used was seawater from Santa Monica Bay, California, filtered through a 0.6-$\mu$m polycarbonate Nuclepore filter (twice) to remove protists. The growth medium was cell-free filtered seawater made two different ways: virus-free seawater was made by filtering through a 0.02-$\mu$m pore size Anodisc filter, and seawater containing natural viruses was made by filtering through a 0.2-$\mu$m Nuclepore filter. The cultures grew within 1–2 days and were observed for 5 days. Bacterial community composition was monitored by a genetic fingerprinting method called terminal restriction fragment length polymorphism (TRFLP), which provides a list of operational taxonomic units based upon variations in 16S rRNA sequences (Avaniss-Aghajani *et al.*, 1994). The bacterial primers and *Hha* 1 restriction enzyme we used were from Gonzalez *et al.* (2000), who also discuss the interpretation of this sort of TRFLP.

The data in Figure 1 demonstrate that the presence of viruses apparently reduced the dominance of a few taxa, particularly the most abundant one that was presumptively identified as marine $\alpha$ Proteobacteria related to *Roseobacter*. The TRFLP peak representing this group dropped significantly, although even without viruses it was the peak with the most amplified DNA. Several taxa, as indicated by other TRFLP peaks, were detectable in only one or the other treatment, grown with or without the presence of viruses. Therefore, the viruses appeared to have an impact on bacterial community composition, helping some groups and hurting others. Although most of the same common taxa were present in both treatments, it should be noted that the experiment lasted only a few generations. Therefore, the relatively subtle effects we observed on a time scale of days would be amplified considerably over weeks or months. Therefore, the data are consistent with the "kill the winner" hypothesis.

Virus-mediated genetic exchange occurs by a process known as transduction. It has been shown to occur in natural environments—for example, freshwater (Ripp *et al.*, 1994) and marine habitats (Jiang and Paul, 1998)—as detected by transfer of selectable markers. Preliminary calculations show that, considering high bacterial abundance, it can be a frequent event: Jiang and Paul (1998) estimated $10^{14}$ transduction events per year in Tampa Bay alone. Despite this high potential, genes transferred this way in natural envi-

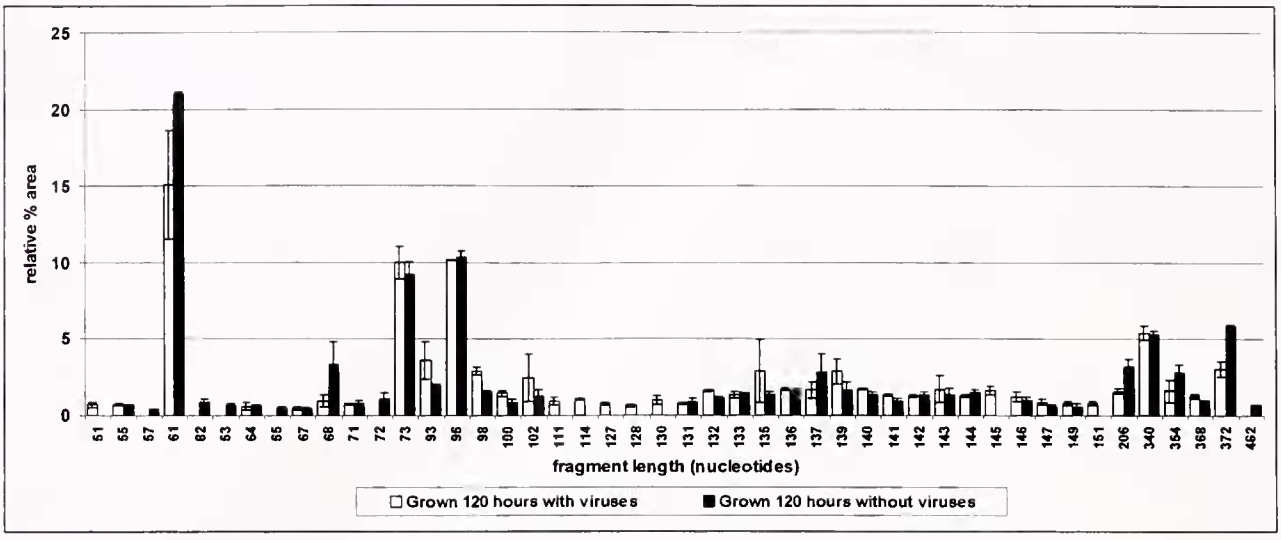


**Figure 1.** Analysis, by terminal restriction fragment length polymorphism, of bacterial community composition in seawater inocula (filtered to remove protists) grown in seawater that had been filtered to remove all cellular organisms and viruses ("without viruses") or cellular organisms only ("with viruses"). Each bar represents an operational taxonomic unit, and its height is proportional to the amount of amplified DNA in that particular fragment. Error bars are ± one standard error of the mean.

ronments are not characterized, and we know very little about the overall process.

Virus effects on bacterial evolution can stem from both negative and positive interactions. Negative interactions include infection-mediated mortality that can provide selective pressure. Resistance of hosts and viral adaptation to resistance is probably an ongoing process—it can be viewed as a "war," with measures and counter-measures and constant adaptation of both partners. It would seem that viruses are unlikely to lead to extinctions, because as the host population size got smaller, the viruses would have more and more difficulty in "finding" a host by diffusion. Nevertheless, a local reduction in population size might cause an evolutionary bottleneck. Positive interactions include viral conversion whereby viral genetic material codes for new host capabilities. Also, bacteria may use viruses as a nutrition source, maybe even using "decoy" receptors to lure "unsuspecting" viruses to attempt infection (Fuhrman, 1999). There are obviously additional evolutionary effects of genetic exchange mediated by viruses. On evolutionary time scales, this process helps to homogenize genomes, especially within close relatives, which may help to keep a bacterial "species" together.

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